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Pathogenicity of local isolates of entomopathogenic fungi against *Spilarctia oblique* at Pantnagar

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Abstract
The aim of the present study is to test the pathogenicity of different entomopathogenic fungal isolates against Bihar Hairy Caterpillar (*Spilosoma obliqua*). On the basis of preliminary bio efficacy trials on all local isolates, two most virulent isolates of each *Beauveria bassiana*, *Metarrhizium anisopliae* and *Nomuraea rileyi* were selected for bio efficacy studies against *S. obliqua*. The results on dose mortality response of *S. obliqua* to two isolates of *B. bassiana* indicated that the LC_{50} values of first isolate were lower than the second isolate indicating that isolate 1 as more virulent. Similarly the first isolate of *M. anisopliae* was found to be more virulent to *S. obliqua* with a lower LC_{50} value, Relative LC_{50} values between two isolates showed that second isolate was 17.8, 50 and 4.1 times more virulent than the first isolate for 4-5, 10-11 and 15-16 days old larvae. Isolate 1 of *N. rileyi* was more effective with lower LC_{50} values. *S. obliqua* was found to be most susceptible to *B. bassiana* followed by *M. anisopliae* and *N. rileyi* respectively.

Keywords: Entomopathogenic fungi, beauveria bassiana, metarhizium anisopliae, nomuraea rileyi

Introduction
Spilarctia obliqua Walker (Lepidoptera: Arctiidae), commonly known as Bihar hairy caterpillar is a polyphagous and sporadic pest attacking nearly 126 plants species distributed in 24 families [13]. This pest has been reported to cause extensive damage to crops such as oilseeds, pulses, vegetables, fodder, fiber crops, fruit trees [12]. The pest falls under the category of key pests and is responsible for the loss in various crops. However the entomopathogenic fungi have been known to cause infection and mortality in this pest of national importance.

The entomopathogens are omnipresent microorganisms attacking various arthropods by causing acute infection. The entomopathogenic microorganisms have the ability of spreading vigorously with the help of conidia by penetrating the cuticle with germ hyphae. Soon after the entry draw nutrition from the body fluids, depleting nutrients from degraded proteins and fat bodies, and produce toxins which kill the host with the help of toxins. After the host's death, the mycelium grows throughout the cadaver and protrudes outside completing the life cycle by abundant conidial formation [4]. Many strains of entomopathogenic fungi have been isolated and tested on different insect pests in a variety of cropping systems [7]. To date, several fungal strains have been successfully licensed for commercial use against whiteflies, aphids, thrips and numerous other [11]. Pathogenicity of the entomopathogenic fungi refers to their ability and inherent capacity to cause disease in insects. This ability of these organisms can be exploited artificially to manage the insect pest problem in an environment friendly way. In order to use these microbes as biological control agents we need to know their virulency and the extent to which they can inflict infection in the key pests. Fungal agents are among the most promising group of biological control agents against insect pests [9]. Over 500 species of fungi are known to have insect pathogenic properties. Interestingly, *Beauveria* and *Metarhizium* (Deuteromycotina, Hyphomycetes) represent the most frequently used genera [1]. Thus in the present study the comparative pathogenicity of the local isolates of entomopathogenic fungi to *S. obliqua* were assessed. Such research holds a lot of scope for the future as better isolates can be identified and isolated and checked on the basis of their pathogenicity and virulence. Such isolates can further be used in the future years to come to manage the pest problem in an ecofriendly manner.

Materials and Methods

The insect used for this experiment was reared in the laboratory in Department of entomology. Different instars of *S. obliqua* were collected from Norman. E. Bourloug Crop Research Centre G.B. Pant University of Agriculture and Technology. The lepidopterous test insects were reared on the preferred host in the laboratory at room temperature (27±4 °C) and 70% relative humidity. The fungal bioassay trials were then conducted on them.

Culture of *S. obliqua*

Different instars of *S. obliqua* were collected from Crop Research Centre. They were then transferred into round glass jars (20x16 cm) containing disinfected fresh leaves of castor *Ricinus communis* (Linn.). The jar was then covered with a moist muslin cloth which was secured in its position with the help of a rubber band. The number of larvae per jar varied according to the instars (100 for first and second, 50 for third and 12 for fourth and later instars). The larvae were transferred everyday into clean disinfected jars. The pupae were kept for adult emergence separately. The adults were transferred to the oviposition jars lined with filter paper and cotton plug soaked in 15 percent sucrose solution was provided as food for the adult moths. The top of the jar was covered with muslin cloth. The eggs laid by female adult were kept in plastic boxes (10cm x 10cm x 14cm). In order to provide proper humidity, a lining of wet filter paper was kept at the bottom. Eggs were incubated at 28±10C and 80±5% relative humidity for hatching. Larvae of 4-5, 10-11, 14-15 days old were used for conducting the experiments.

Harvesting of conidia and conidial count

For the bioassay tests, conidia were harvested just before use from one month old culture by washing from the surface of the plates using 100 ml of sterilized distilled water containing 0.02-0.05% Tween 80 under aseptic conditions by scrubbing with a sterile infection loop [10]. The viability of conidia was determined prior to application as suggested by Gillespie (1986) [3]. Different concentrations of conidia were prepared for each isolate after assessing the number of conidia in the suspension with an improved Neubauer Weber Haemocytometer [5].

Bioassay trials of entomopathogenic fungus against *S. obliqua*

For bioassay trials the larvae of *S. obliqua*, were collected from the nucleus culture maintained in the laboratory. Six concentration of spore suspension of fungal pure cultures were used for the test. The experiment was carried out on 4-5, 10-11 and 15-16 days old larvae of test insects. The conidia for the bioassay test were harvested from 18 day old cultures and suspension was made in distilled water containing 0.02 per cent tween 80. Ten larvae taken in a petriplate (9 cm dia.) lined with a filter paper were sprayed directly with 2-3 ml conidial suspension using a hand atomizer. Three replications were maintained for each concentration. Sterile distilled water containing 0.02 per cent Tween 80 was sprayed on the insects which served as control. After air drying, treated larvae were carefully transferred to individual sterile round plastic vials (4.5×12cm) containing fresh pieces of castor leaves that were washed with sterilized distilled water. The vials with screw caps having provision for proper aeration for the larvae were maintained in an incubator set at 27±1 ^{0}C and 95±5 percent relative humidity. For 4-5 and 10-11 day age group, initially 10 larvae were reared in a plastic vials. After 5 days of spraying they were reared individually until the experiment was terminated. The leaves were first changed after 48 h of spraying. Thereafter, the fresh leaves were provided. The experiment was replicated three times and ten larvae were reared in each replication. The observations on mortality were taken at 24 h interval upto pupation. Pathogenicity of different isolates of *B. bassiana*, *M. anisopliae* and *N. rileyi* were studied on different age group of test insect. Computation of LC_{50} was based on that at which maximum cumulative mortality in a concentration occurred. Observations on mortality were subjected to Probit analysis [2].

Results and Discussion

Susceptibility of *S. obliqua* to fungal isolate

Dose mortality relationship of *B. bassiana on S. obliqua* has been assessed in this research. For understanding the effectiveness of different isolates of the fungus LC_{50} was calculated along with their fiducial limits. These values were computed on the basis of maximum cumulative mortality in a day. The results on dose mortality response of *S. obliqua* to two isolates of *B. bassiana* indicated that isolate 1 was more virulent with LC_{50} values of 1.41 x 10^5, 6.84 x 10^5 and 3.9 x 10^8 conidia/ ml for 4-5, 10 – 11 and 15- 16 day old larvae respectively than the isolate 2 of *B. bassiana* where L.C_{50} value of 8.6 x 10^6, 8.7 x 10^8 and 1.0 x10^9 conidia/ ml were observed (Table 01). Relative LC_{50} values for 10- 11 and 15-16 day old larvae were 4.85 and 2765 times more than 4-5 days old larvae of isolate 1 For second isolate the relative LC_{50} was 101 and 116 times more than 4-5 day old larvae. Relative LC_{50} between the two isolates showed that the relative LC_{50} values of first isolate were 60.9, 1271 and 2.56 times more than the second isolate for 4-5, 10-11 and 15-16 day old larvae respectively. Thus there was difference in the virulency of the two isolates. Dose mortality response of two isolates of *M. anisopliae* against *S. obliqua* is presented in Table 01 LC_{50} value of 4-5, 10-11 and 15-16 day old larvae of isolate 1 of *M. anisopliae* were 2.3 x 10^5, 1.8 x 10^6, 7.3 x 10^8. While in the case of second isolate the LC_{50} values were 4.1 x 10^6, 9.0 x 10^7 and 3.0 x 10^9 conidia/ml for 4-5, 10-11 and 15-16 day old larvae respectively. The results show that isolate 1 was more effective with lower LC_{50} values. Relative LC 50 value 10-11 and 15-16 day old larvae were approximately 7.8 and 3173 times more as compared to 4-5 day old larvae, while for isolate 2, these were 21.95 and 731.7 times more. Relative LC 50 values between two isolates showed that LC 50 value of second isolate were 17.8, 50 and 4.1 times more than the first isolate for 4-5, 10-11 and 15-16 days old larvae this shows that the two isolates were not at power in inducing mortality in *S. obliqua.* Dose mortality response of two isolates of *N. rileyi* against *S. obliqua* is presented in Table 01. LC_{50} value of 4-5, 10-11 and 15-16 day old larvae of isolate 1 of *N. rileyi* were 9.5 X 10^7, 3.9 X 10^9, 1.3 X 10^{10} respectively While in the case of second isolate the LC_{50} values were 5.1 X 10^9, 8.8 X 10^{10}, 6.7 X 10^{11} conidia per ml for 4-5, 10-11 and 15-16 day old larvae respectively. The results show that isolate 1 is more effective with lower LC_{50} values. Relative LC_{50} value 10-11 and 15- 16 day old larvae were approximately 41.0 and 136.84 times more as compared to 4-5 day old larvae, while in isolate 2, these were 17.2 and 131.3 times more. Relative LC_{50} values between two isolates showed that LC_{50} value of second isolate were 53.6, 22.5 and 51.5 times more than the first isolate for 4-5, 10-11 and 15-16 days old larvae this shows that the two isolates had marked

difference in terms of inducing virulence.

The results show that the various isolates of *B. bassiana, M. anisopliae* and *N. rileyi* differed from each other in terms of virulence to *S. obliqua*. The variation in LC_{50} values may occur due to fungal strains and species of a given insect and the mode of contamination [6]. Loc (1995) [8] also confirmed the findings and emphasized that in *S. obliqua* the thickness of hair increase as the age of the larvae advance, this acts as a physical barrier and hinder the contact of the fungal spores with the integument, this is a probable explanation for the results that observed that the fungi was more effective to the younger stages of *S. obliqua.*

Table 1: Dose mortality response of *B. bassiana, M. anisopliae* and *N. rileyi* to *S. obliqua*

Entomopathogens	Isolates	Age of larvae	χ 2	Y=a+bx	LC_{50}	Feducial limits	Relative LC 50	Relative LC 50
B. bassiana	Isolate 1	4-5 day	1.35	Y= 0.325x +4.061	1.41×10^5	$3.4 \times 10^4 – 5.7 \times 10^5$	1	1: 0.01 1: 1271 1: 0.39
		10-11day	1.31	Y= 0.170x +4.593	6.84×10^5	$3.21 \times 10^3 – 1.4 \times 10^5$	4.85	
		15-16day	0.65	Y= 0.168x +3.961	3.9×10^8	$4.0 \times 10^6 – 3.8 \times 10^{10}$	2765	
	Isolate 2	4-5 day	1.48	Y= 0.385x +3.284	8.6×10^6	$2.2 \times 10^6 – 3.3 \times 10^7$	1	
		10-11day	1.54	Y= 0.345x +2.789	8.7×10^8	$5.1 \times 10^7 – 1.5 \times 10^{10}$	101	
		15-16day	1.39	Y= 0.351x +2.673	1.0×10^9	$6.9 \times 10^7 – 1.5 \times 10^{10}$	116	
M. anisopliae	Isolate 1	4-5 day	0.848	Y = 0.224x + 4.32	2.3×10^5	$3.1 \times 10^4 – 1.7 \times 10^6$	1	1: 0.05 1: 0.02 1: 0.24
		10-11 day	0.369	Y = 0.25x + 4.01	1.8×10^6	$3.1 \times 10^5 – 1.0 \times 10^7$	7.8	
		15-16 day	1.598	Y = 0.491x + 1.97	7.3×10^8	$5.6 \times 10^7 – 9.5 \times 10^9$	3173	
	Isolate 2	4-5 day	0.116	Y = 0.187x + 4.19	4.1×10^6	$3.4 \times 10^6 – 2.0 \times 10^9$	1	
		10-11 day	1.249	Y = 0.211x + 3.80	9.0×10^7	$3.9 \times 10^6 – 2.0 \times 10^9$	21.95	
		15-16 day	0.483	Y = 0.257x + 3.15	3.0×10^9	$4.2 \times 10^7 – 2.1 \times 10^{11}$	731.7	
N. rileyi	Isolate 1	4-5 day	0.122	Y= 0.261x +3.471	9.5×10^7	$5.8 \times 10^6 – 1.5 \times 10^9$	1	1: 53.6 1: 22.5 1: 51.5
		10-11 day	0.176	Y = 0.222x+3.361	3.9×10^9	$1.8 \times 10^7 – 8.3 \times 10^{11}$	41.0	
		15-16 day	0.105	Y = 0.213x +.313	1.3×10^{10}	$2.2 \times 10^7 – 7.9 \times 10^{12}$	136.84	
	Isolate 2	4-5 day	0.388	Y = 0.236x+3.443	5.1×10^9	$1.1 \times 10^7 – 2.4 \times 10^{10}$	1	
		10-11 day	0.408	Y = 0.191x+3.312	8.8×10^{10}	$2.0 \times 10^7 – 3.8 \times 10^{14}$	17.2	
		15-16 day	0.699	Y = 0.194x +3.204	6.7×10^{11}	$1.2 \times 10^7 – 3.5 \times 10^{16}$	131.3	

Conclusion

The present study found that *S. obliqua* was most susceptible to *B. bassiana* followed by *M. anisopliae* and *N. rileyi* respectively. The local isolates showed promising results. They were able to cause infection in the test insect *S. obliqua.* Both the isolates showed virulence against the test insects.

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